



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US00/02730 (22) International Filing Date: 02 February 2000 (02.02.2000) (30) Priority Data: 09/243,018 03 February 1999 (03.02.1999) US (60) Parent Application or Grant WIKTOR, Peter [/]; (). WIKTOR, Peter [/]; (). GRAY, Richard, O., Jr. ; ().		<b>Published</b>
(54) Title: <b>PIEZOELECTRIC PIPETTING DEVICE</b> (54) Titre: <b>DISPOSITIF DE PIPETTAGE PIEZOELECTRIQUE</b>		
(57) Abstract		
<p>A piezoelectric actuated device for acquiring and dispensing fluid samples is described. Fluid samples are acquired or drawn up into the device by dipping the tip into a fluid and applying an alternating current electrical signal to the piezoelectric element. Removing the tip from the fluid and applying another electrical signal causes drops of fluid to be ejected for non-contact dispensing. The device optionally incorporates a second piezoelectric element functioning as a sensor to detect if it is empty, plugged or working properly. An optional heater, temperature sensor and feedback circuitry regulate the temperature of the device.</p>		
(57) Abrégé		
<p>L'invention concerne un dispositif piézoélectrique d'acquisition et de distribution d'échantillons liquides. Des échantillons liquides sont prélevés ou aspirés dans le dispositif par introduction de la pointe dans un liquide et application d'un signal électrique de courant alternatif à l'élément piézoélectrique. Le retrait de la pointe du liquide et l'application d'un autre signal électrique provoquent une éjection de gouttes du liquide permettant une distribution sans contact. Le dispositif incorpore facultativement un second élément piézoélectrique qui fonctionne comme un capteur pour détecter s'il est vide, bouché ou fonctionne correctement. Un appareil de chauffage, un capteur de température et un circuit de réaction régulent la température du dispositif.</p>		

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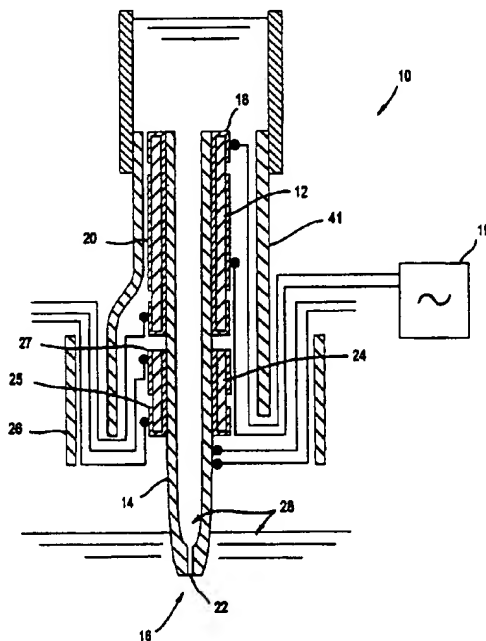
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(21) International Application Number: PCT/US00/02730 (22) International Filing Date: 2 February 2000 (02.02.00) (30) Priority Data: 09/243,018 3 February 1999 (03.02.99) US (71)(72) Applicant and Inventor: WIKTOR, Peter [US/US]; 3226 Northeast 87th Street, Seattle, WA 98115 (US). (74) Agents: GRAY, Richard, O., Jr. et al.; Graybeal Jackson Haley LLP, Suite 350, 155 108th Avenue Northeast, Bellevue, WA 98004-5901 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.

(54) Title: PIEZOELECTRIC PIPETTING DEVICE

(57) Abstract

A piezoelectric actuated device for acquiring and dispensing fluid samples is described. Fluid samples are acquired or drawn up into the device by dipping the tip into a fluid and applying an alternating current electrical signal to the piezo-electric element. Removing the tip from the fluid and applying another electrical signal causes drops of fluid to be ejected for non-contact dispensing. The device optionally incorporates a second piezoelectric element functioning as a sensor to detect if it is empty, plugged or working properly. An optional heater, temperature sensor and feedback circuitry regulate the temperature of the device.



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Description

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**PIEZOELECTRIC PIPETTING DEVICE****BACKGROUND OF THE INVENTION**

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5 The present invention relates to a piezoelectric actuated device for manually or automatically transferring very small volumes of fluid. Transferring fluids from one container to another is one of the most common tasks performed in a typical chemical or biological laboratory. For example, various chemicals from different containers may have to be mixed together and then the mixture may have to be divided out equally or 'aliquoted' into other containers or onto microscope slides or some other substrates. Currently transferring of fluids is done either by hand using manual pipettors or automatically using an automated robotic pipetting instruments. Motivated by the high costs of chemical reagents there is a trend in chemical and biological laboratories to perform experiments using smaller and smaller fluid samples.

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15 Prior to the 1970's fluid transfer was done in laboratories by hand using glass tubes called pipettes. Samples were aspirated by sucking on the end of the pipette by mouth like a straw and then sealing the end with a finger. Fluid samples were then dispensed or aliquoted by briefly unsealing and then resealing the pipette with the finger allowing a small volume of fluid to flow out. This method required a fair amount of dexterity and had some serious drawbacks in terms of ergonomics, precision and health risks. During the same time period glass capillaries were also used for transferring smaller volumes of fluid with greater precision. These techniques for manually transferring fluid samples were made obsolete in the early 1970's by the introduction of the manual pipette as described, for example, in U.S. Patent Nos. 3,613,952, 3,766,785, 3,827,305 and 3,991,617, with ejectable, disposable plastic tips. Currently the manual pipette is a ubiquitous and indispensable tool for transferring fluid samples in the modern laboratory.

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Although manual pipetting is a big improvement over earlier techniques it is tedious for the human operator and can cause repetitive motion injuries as well as being prone to human error. Currently, a great variety of automated pipetting instruments are commercially available which address these specific drawbacks.

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5 These instruments, as advanced by Tecan AG of Hombrechtikon Switzerland,  
typically use a Cartesian X,Y,Z robot to move a pipetting head among various  
aspirating, dispensing and washing stations. The various types of instruments  
10 currently available differ mainly in the mechanisms used to acquire and then  
5 dispense fluid samples. The most common mechanism for fluid transfer is the  
syringe pump as used in instruments manufactured by Tecan, Hamilton Company,  
Cavro Scientific Instruments, Robbins Scientific, Qiagen and Tomtec among others.  
15 In most cases the syringe pumps and pipette tips are connected via long flexible  
tubing, however, in the instruments supplied by Tomtec and Robbins Scientific the  
10 syringe pumps are mounted directly to the back of the pipette tips. The smallest  
volume that can be accurately transferred using syringe pumps is approximately 0.1  
20 microliters. U.S. Patent. Nos. 5,743,960 and 5,741,554 describe an instrument  
which combines a syringe pump with a solenoid valve allowing drops as small as 0.1  
microliters to be ejected onto a substrate for non-contact printing or arraying  
25 applications. By contrast, the drops that are dispensed using the present invention  
are a thousand times smaller.

Another method for transferring small volumes of fluid for arraying  
30 applications is the use of pins as described in U.S. Patent No. 5,807,522.  
Instruments using pins for fluid transfer are used by Synteni, among other  
20 companies, to generate DNA arrays and are commercially available, for example,  
from BioRobotics and GeneMachines. Using pins is a simple, robust and practical  
35 means for fluid transfer but it suffers from some limitations. First of all it is slow. The  
pins have to stop at each spot and then wait for over a second for capillary action to  
transfer the fluid onto the substrate. By contract, the piezoelectric based dispensing  
25 of the present invention is almost a thousand times faster. Pin based fluid transfer is  
sensitive to the wetting properties of the substrate. Also it can damage some  
40 substrates like Nylon membranes for example. These are not concerns for the  
piezoelectric dispensing which is non-contact. Pins generate relatively low density,  
45 poor quality arrays with approximately 50% variability in spot size. By contrast,  
30 piezoelectric dispensers generate arrays with almost an order of magnitude higher  
density and better than 3% spot size variability. Finally, pins are limited to acquiring

5 and dispensing a fixed volume of fluid. Piezoelectric dispensers have thousands of  
times higher dynamic range. Sub nanoliter to tens of microliter volumes can be  
aspirated and subsequently dispensed in volumes ranging from 100 picoliters to  
10 several microliters per second.

5 A number of companies including Microdrop, Packard Instruments and  
GeSiM, use piezoelectric fluid dispensing devices to dispense drops of fluid with  
volumes on the order of 100 picoliters. Originally piezoelectric dispensing  
15 technology was used for "drop-on-demand" "ink-jet" printing. These devices as  
described in U.S. Patent No. 2,512,743 have a fluid filled chamber with an inlet at  
10 one end and a nozzle on the other. A piezoelectric element induces an acoustic  
wave in the fluid causing a drop to be ejected from the nozzle. For printing  
20 applications ink is supplied to the back end of the piezoelectric fluid dispenser from a  
reservoir. For fluid transfer applications fluid is drawn up through the nozzle. The  
instruments supplied by Microdrop, Packard Instruments and GeSiM all use syringe  
25 pumps to aspirate fluids up through the nozzle of the piezoelectric devices. Syringe  
pumps impose several serious limitations on current piezoelectric based pipetting  
instruments. It turns out that properly aspirating samples prior to dispensing is one  
30 of the most critical considerations for reliable operation of piezoelectric fluid  
dispensers. Syringe pumps can sometimes aspirate air bubbles and small particles  
20 that can adversely affect the dispensing characteristics. Also, variations in back  
pressure in the flexible tubing leading to the syringe pumps causes the drop-on-  
demand dispensing properties to change. Packard Instruments uses a closed loop  
35 pressure controller to actively regulate this back pressure. This system adds to the  
cost and complexity of the instrument. Additionally the syringe pumps themselves  
25 add significantly to the cost, size and complexity of the instrument and they limit the  
smallest fluid volumes that can be transferred to around 0.5 microliter. By contrast,  
40 with the present invention, volumes over 100 times smaller can be aspirated. Most  
importantly, aspirating is much more uniform and precise making dispensing more  
45 reliable and repeatable. In the present invention, the dispensers are opened to  
30 ambient pressure so the back pressure always remains uniform. Additionally the  
novel sensing technology of the present invention detects almost instantly if there is

5 a problem with the dispensers, e.g. if they are clogged, empty or attempting to aspirate from an empty well.

10 The present invention provides improvements to the cylindrical piezoelectric fluid dispenser described in U.S. Patent No. 3,840,758. These improvements  
5 enable bi-directional operation of the device, sensing of the operational state and thermal control. As a summary of some related devices. Humberstone in U.S. Pat. No. 5,518,179 describes a bi-directional device with a piezoelectric driven, thin,  
15 perforated membrane capable of drawing a fluid up through the perforations as well as dispensing fluid out through them. Beckman et al. in U.S. Pat. No. 4,432,699  
20 describes a peristaltic piezoelectric pump with an internal load sensor. Hayes in U.S. Pat. No. 5,622,897 describes a process of manufacturing a drop-on-demand ink-jet print head having n-type and p-type thermoelectric carriers.

#### **SUMMARY OF THE INVENTION**

25 15 The invention provides a device enabling the automatic or manual transfer of small volumes of fluid from one container to another or from one container to the surface of a substrate. The device is a self contained, bi-directional pump in the  
30 sense that it can acquire fluid samples as well as dispense them. Acquiring samples in accordance with the present invention requires dipping the tip of the device into  
20 the sample fluid. Dispensing, however, does not require any contact with either a fluid or solid surface. Fluid can be dispensed one drop at a time in a "drop-on-demand" mode or continuously at frequencies from over several thousand to fifty  
35 thousand drops per second.

40 25 In accordance with further aspects of the present invention, an integral monitoring sensor detects if the piezoelectric pipette is functioning normally, is empty or if the nozzle is plugged. This type of feedback of the operational state of the device is critical for automated pipetting instruments to alert a human operator if the device requires some attention or to initiate an automated failure recovery  
45 procedure.

50 30 The characteristics of drop-on-demand ink-jet dispensers can change with temperature. In accordance with a further aspect of the present invention, a



5 piezoelectric pipette incorporates a built in temperature regulator. This helps  
maintain uniform dispensing characteristics even if the ambient temperature  
changes. The temperature regulator can also be used to raise the temperature of  
10 the fluid being dispensed. Elevated temperatures can reduce the viscosity of fluids  
5 that are too viscous to dispense at ambient temperature.

In summary, the invention is a compact, bi-directional pump for acquiring and  
dispensing small volumes of fluid with a built in monitoring sensor and temperature  
15 control. The invention is suitable for automated or manual fluid transfer applications.

Accordingly, the invention provides a diffuser valve pump including a pipette  
10 having a capillary having a first end including a nozzle and a piezoelectric actuating  
element adjacent to the capillary and an alternating current voltage source coupled  
20 across the piezoelectric actuating element. With the nozzle immersed in a sample  
fluid and the capillary at least partially filled with a working fluid, alternating current  
voltage applied to the actuating element causes sample fluid to be drawn into the  
25 15 capillary.

The invention further provides a pipette sensing arrangement for sensing a  
working condition of a pipette having a capillary with an end including a nozzle, a  
piezoelectric actuating element adjacent to the capillary, and first electrodes for  
30 applying electrical energy across the piezoelectric actuating element. The sensing  
20 arrangement includes a piezoelectric sensing element adjacent to the capillary and  
second electrodes for coupling the piezoelectric sensing element to a voltage  
35 sensor.

The invention still further provides a temperature control arrangement for a  
pipette having a capillary having an end including a nozzle, a piezoelectric actuating  
25 element adjacent to the capillary, and actuating electrodes for applying electrical  
40 energy across the piezoelectric actuating element. The temperature control  
arrangement includes a current source for passing an electrical current through one  
of the actuating electrodes, and a temperature sensor closely adjacent the capillary  
45 for sensing the temperature of the capillary.

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**BRIEF DESCRIPTION OF THE DRAWINGS**

The features of the present invention which are believed to be novel are set forth with particularity in the appended claims. The invention, together with further objects and advantages thereof may best be understood by making reference to the following description taken in conjunction with the accompanying drawings, in the several figures of which like reference numerals identify identical elements, and wherein:

Figure 1 is a cross-sectional view of piezoelectric pipette embodying the present invention including a sensor, temperature controller, Teflon housing and reservoir;

Figure 2 is a cross-sectional view of piezoelectric pipette in a diffuser valve pump mode in accordance with the present invention with a reservoir attached to the back end and a mounting bracket arm for automated dipping into the sample fluid;

Figure 3 is a manually operated piezoelectric pipette;

Figure 4 is a cross-sectional view of piezoelectric pipette embodying further aspects of the present invention with a tube attached to the back end and another tube attached to the front end;

Figure 5 is a cross-sectional view of two piezoelectric pipettes back-to-back forming a reversible continuous flow pump in accordance with the present invention;

Figure 6 is a cross-sectional view of piezoelectric pipette with a separate sensor embodying the present invention;

Figure 7 is a graph of the power spectral densities of the sensors for water vs. Fluorinert fluids with the device operating in drop-on-demand mode illustrating a further aspect of the present invention;

Figure 8 is a block diagram of a dispenser actuation and sensor detection system embodying the present invention;

Figure 9 is a flow diagram illustrating an actuation and detection method embodying the present invention;

Figure 10 is a cross-sectional view of a piezoelectric pipette with a thermocouple and heating system including an inner electrode functioning as an integrated heating element embodying the present invention; and

Figure 11 is a block diagram of a temperature control system which may be employed with the pipette of Figure 10 in accordance with the present invention.

#### **DETAILED DESCRIPTION**

Referring now to Figure 1, it illustrates a piezoelectric pipette 10 with sensor, temperature control, housing and reservoir embodying the present invention. It includes an actuating piezoelectric ceramic tube 12 with a glass capillary 14 bonded concentrically on the inside. The glass capillary has a nozzle 16 formed on one end by heating and then polishing the end. Applying a voltage to the piezoelectric crystal 12 across an inner electrode 18 and an outer electrode 20 causes it to deflect which induces an acoustic wave in the fluid filled glass capillary 14. A drop is ejected from the nozzle if the acoustic energy is high enough to overcome surface tension at the nozzle opening 22. In accordance with the present invention, a separate sensing piezoelectric ceramic tube 24 is concentric with the glass capillary 14 and adjacent to the actuating piezoelectric tube 12. These elements may also be seen in Figure 6 with the corresponding elements having corresponding reference numerals with an "a" subscript. The piezoelectric pipette is housed in Teflon heat shrink tubing 26 and 41 as shown in Figure 1. This housing is compact, provides mechanical protection and electrical insulation for the piezoelectric crystal.

#### **Diffuser Valve Pump Mode**

With the nozzle immersed in fluid 28 as shown in Figure 1 the device 10 operates as a diffuser valve pump capable of acquiring or drawing fluid samples up through the nozzle. Due to the shape of the nozzle, fluid has less resistance flowing from the sample fluid up into the glass capillary than out. Periodically deflecting the piezoelectric crystal by applying an alternating voltage from an alternating current source 19 to the electrodes 18 and 20 causes fluid to flow in and out of the nozzle with a net inward flow due to the lower flow resistance in this direction. Based on the diffuser valve pump principle it is possible to draw up fluid samples into the piezoelectric pipette and then to dispense them as a drop-on-demand 'ink-jet' printer.

5 The diffuser valve pump works over a large range of periodic excitation  
voltage frequencies, amplitudes and waveforms. Pumping speed increases with  
frequency and amplitude. This diffuser valve pump mode has been demonstrated to  
10 successfully pump fluid with excitation signal frequencies ranging from 6 KHz to 25  
5 KHz and amplitudes from 20 V to over 90 V. Pumping was demonstrated with a  
variety of waveform shapes including saw-tooth, square-wave and sine-wave  
waveforms.

15 The glass capillary of the device must be at least partially filled with fluid for  
both drop-on-demand and diffuser valve pump modes to function properly. This  
20 'working' fluid does not necessarily have to be the same as the sample fluid as  
illustrated in Figure 2 which shows another pipette 10b wherein corresponding  
elements are designated by corresponding reference numerals with a "b" subscript.  
Here the working fluid 30 and the sample fluid 28b are different. The working and  
sample fluids may be immiscible with each other allowing small fluid samples to be  
25 drawn up without diffusing the sample into the working fluid. A wide variety of fluids  
immiscible in, for example, water in practicing the present invention may be selected  
including Fluorinert manufactured by 3M, 200 Fluid manufactured by Dow Corning  
and paraffin. Fluorinert has a good combination of properties for many applications  
30 including low viscosity, good bio-compatibility and it does not leave a residue when  
20 dried out.

35 As also illustrated in Figure 2, the piezoelectric pipette 10b can be mounted  
on a supporting mounting bracket arm 32. This arm can be attached to an  
automated robotic instrument which dips the device into the sample fluid 28b for  
aspirating and then moves it to another station for dispensing. Alternatively, the  
40 piezoelectric pipette 10b with wires 33 can be attached to a manual pipettor 34  
having a disposable tip 36, as shown in Figure 3. In this case the device is dipped  
into the sample fluid by a human operator and then manually moved to another  
container or substrate for dispensing. The plunger 38 of the manual pipettor can be  
45 used for priming, rinsing and emptying the device.

50 Three different versions of the piezoelectric pipette 10b operating as a  
diffuser valve pump are shown in Figure 2, Figure 4 and Figure 5. As seen in

5 Figure 2, attaching a reservoir 40 to the back end 42 of the piezoelectric pipette 10b  
allows larger volumes of fluid 28b to be drawn up. As seen in Figure 4, by attaching  
10 tubing 44 and 46 to respective ends 48 and 42b respectively of pipette 10b, the  
piezoelectric pipette operates as a pump for a continuous flow system. A reversible  
5 continuous flow pump can be made by putting two piezoelectric pipettes 10b back to  
back as shown in Figure 5. In each of the embodiments of Figures 2 and 4, the  
15 voltage source 19 of Figure 1 may be used to apply an alternating current voltage  
across the inner and outer electrodes of the actuating piezoelectric elements. In the  
embodiment of Figure 5, separate voltage sources may be used for the actuating  
10 electrodes or a single source may be selectively applied to one or the other of the  
actuating elements.  
20

#### Integrated Sensor

25 The devices 10 and 10a shown in Figure 1 and Figure 6 respectively each  
15 incorporate a separate piezoelectric element 24 and 24a respectively which  
functions as a sensor to monitor the operational state of the device. The sensing  
elements 24 and 24a are proximal to their corresponding actuating elements 12 and  
30 12a respectively with respect to the nozzles 22 and 22a. The sensor 24, 24a  
detects vibrations induced in the fluid 28, 28a, the glass capillary 14, 14a and the  
20 actuating piezoelectric crystal 12, 12a. By analyzing the voltage signal generated by  
this sensor across electrodes 25 and 27 and 25a and 27a it is possible to identify the  
35 operational state of the device, specifically, if the dispenser is operating properly  
both in drop-on-demand and diffuser valve pump modes of operation. Specifically it  
is possible to detect if the device is working properly, if it is empty or full when  
40 25 aspirating or dispensing, if the nozzle is partially or completely plugged when  
aspirating or dispensing, what type of fluid is being dispensed or if the device is  
trying to aspirate fluid from an empty container. The power spectral densities of the  
time signals are computed to make it easier to distinguish the differences between  
45 these various operating conditions. For example, Figure 7 shows the power  
30 spectral density (PSD) of the output from the sensor while the dispenser is  
dispensing in drop-on-demand mode. The PSDs in Figure 7 were computed by  
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5 averaging the fast Fourier transforms (FFTs) of 16 individual Hanning windowed  
data records of 128 points each sampled at a frequency of 375 KHz. The figure  
shows the difference in the sensor output if water 43 vs. Fluorinert 45 is being  
10 dispensed. At 90 KHz there is a difference of three orders of magnitude between  
5 the PSDs of water 43 and Fluorinert 45. Detection software will compare the PSDs  
at this frequency to determine if the sample fluid (water) or the working (Fluorinert)  
fluid is being dispensed.

15 A block diagram of the dispenser actuation and sensor detection system 50 is  
depicted in Figure 8. It incorporates a digital computer 52, drive electronics 54 for  
10 the actuating piezoelectric element 12, 12a, the piezoelectric pipette 10, 10a itself  
with an integrated sensor 24, 24a and an analog to digital converter (ADC) 56 for the  
20 sensor signal. The computer 52, upon implementing operating instructions in  
accordance with this embodiment of the present invention forms an actuation and  
detection stage 58. A flow diagram 60 of the actuation and detection stage  
25 operation is depicted in Figure 9. First in step 62, an actuating trigger 51 command  
to actuate the device is issued to the drive electronics 54. The drive electronics 54  
in turn generates an actuating voltage 53 to drive the actuating piezoelectric element  
30 12, 12a with a voltage waveform. To obtain the sensor data, for Figure 7 for  
example, the piezoelectric element may be driven with a 60 microsecond, 35 volt  
20 amplitude pulse. The voltage causes the actuating piezoelectric element to deflect  
which induces vibrations in the fluid, the glass capillary and the piezoelectric element  
35 itself. These vibrations are in turn converted to a sensor voltage signal 55 by the  
sensing piezoelectric element 24, 24a. The analog to digital converter (ADC) 56 is  
then, in step 64, triggered to sample and digitize this signal. The computer then in  
40 25 step 66 stores the digitized sensor signal 57 output values. Steps 64 and 66 are  
repeated. When the voltage signal is completely sampled, the data is made  
available for digital signal processing and analysis by the computer. The power  
spectral densities of Figure 7 are then computed in step 68 using any accepted  
45 method of power spectral estimation. The PSD is then evaluated in step 70 by  
30 comparing the PSD value at different frequencies to known corresponding values for  
a particular operational state of the device. For each evaluation, the operational

5 state of the device 10, 10a is determined in accordance with step 72. Steps 70 and 72 are repeated for all of the various operational states of the device.

#### 10 Temperature Control

5 Figure 10 shows a piezoelectric pipette 70 wherein the temperature of the glass capillary 72 and thus the fluid 74 within the capillary may be controlled in accordance with a further aspect of the present invention. Here, the actuating element 76 is associated with an outer electrode 78 and an inner electrode 80 having two wrap-around electrode portions 82 and 84 on each end of the  
10 piezoelectric crystal 76. Applying a current between the wrap-around electrode portions 82 and 84 and thus through the inner electrode 80 causes the temperature of the device to increase. A thermocouple 86, depicted in Figure 10, is closely adjacent the nozzle 87 and senses the temperature of the device. The temperature may be regulated with a temperature regulator 88 (Figure 11) such as an Analog  
15 Devices AD594 as specified in the *Monolithic Thermocouple Amplifier Data Sheet* from Analog Devices. This integrated circuit controls the current passing through the inner electrode to maintain a set-point temperature using feedback from the thermocouple as depicted in the block diagram in Figure 11.

20 As can be seen in Figure 11, the temperature regulator 88 provides a control output to a heater driver 90 which applies the current to the inner electrode 80. The amount of current applied is dictated by the temperature regulator 88 and more specifically the difference between the output voltage of the thermocouple 86 and a set point reference voltage applied to a subtractor stage 92.

## Claims

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5                   What is claimed is:

1. A diffuser valve pump comprising:

          a pipette including a capillary having a first end including a nozzle and

10           a piezoelectric actuating element adjacent to the capillary; and

5           an alternating current voltage source coupled across the piezoelectric  
          actuating element, whereby

15           with the nozzle immersed in a sample fluid and the capillary at least  
          partially filled with a working fluid, alternating current voltage applied to the  
          actuating element causes sample fluid to be drawn into the capillary.

10           2. A diffuser valve pump as defined in claim 1 wherein the alternating current  
20           voltage source provides alternating current voltage having one of a saw-tooth  
          square-wave and sine-wave waveform.

25           3. A diffuser valve pump as defined in claim 1 wherein the capillary has a  
15           second end opposite the first end and wherein the diffuser valve pump further  
          includes a reservoir attached to the second end of the capillary.

30           4. A diffuser valve pump as defined in claim 1 wherein the capillary has a  
20           second end opposite the first end and wherein the diffuser valve pump further  
          includes a first conduit attached to the first end and a second conduit attached to the  
35           second end whereby, with fluid filling the capillary and the conduits, the valve pump  
          functions as a continuous flow pump.

40           5. A diffuser valve pump as defined in claim 1 wherein the capillary includes  
25           a second end opposite the first end, wherein the diffuser valve pump further includes  
          a second pipette including a capillary having a first end including a nozzle and a  
          second end opposite the first end and a piezoelectric actuating element adjacent the  
45           capillary, wherein the second ends of pipettes are attached for communicating with  
30           each other, and wherein the diffuser pump valve further includes conduit means  
          attached to the first ends of the pipettes, whereby, with alternating current voltage

5 selectively applied across the actuating elements, and with fluid filling the capillaries  
and conduits, the diffuser valve pump is arranged to function as a reversible  
continuous flow pump.

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5 6. A diffuser valve pump as defined in claim 1 further including a mounting  
arm for disposing the nozzle immersed in a sample fluid.

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7. A pipette sensing arrangement for sensing a working condition of a pipette  
having a capillary having an end including a nozzle, a piezoelectric actuating  
10 element adjacent the capillary, and first electrodes for applying electrical energy  
across the piezoelectric actuating element, the sensing arrangement comprising:  
20 a piezoelectric sensing element adjacent the capillary; and  
second electrodes for coupling the piezoelectric sensing element to a  
voltage sensor.

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15 8. An arrangement as defined in claim 7 wherein the sensing element is  
proximal to the actuating element with reference to the nozzle.

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9. An arrangement as defined in claim 7 wherein the voltage sensor includes  
20 an analog to digital converter for generating digital data responsive to the sensing  
element.

35  
10. An arrangement as defined in claim 9 further including means for  
analyzing the digital data.

40  
25 11. An arrangement as defined in claim 10 wherein the means for analyzing  
includes means for generating power spectral densities.

45  
12. An arrangement as defined in claim 11 wherein the means for analyzing  
30 further includes comparing means for comparing the generated power spectral

5 densities at preselected frequencies to predetermined power spectral density values corresponding to the preselected frequencies.

10 13. A temperature control arrangement for a pipette having a capillary having  
5 an end including a nozzle, a piezoelectric actuating element adjacent the capillary, and actuating electrodes for applying electrical energy across the piezoelectric actuating element, the temperature control arrangement comprising:  
15 a current source for passing an electrical current through one of the actuating electrodes; and  
10 a temperature sensor closely adjacent the capillary for sensing the temperature of the capillary.  
20

25 14. An arrangement as defined in claim 13 wherein the temperature sensor includes a thermocouple.

15 15. An arrangement as defined in claim 13 wherein the temperature sensor is closely adjacent the nozzle.

30 16. An arrangement as defined in claim 17 further including means for  
20 controlling the current source responsive to the temperature sensor.

35 17. An arrangement as defined in claim 16 wherein the temperature sensor includes means for generating a voltage representative of the sensed temperature and wherein the means for controlling is responsive to a difference between the  
40 25 voltage generated by the temperature sensor and a set point reference voltage.

45 18. An arrangement as defined in claim 13 wherein the actuating electrodes include an inner electrode between the actuating element and the capillary and an outer electrode adjacent the actuating element on a side of the actuating element  
30 opposite the capillary, and wherein the current source is coupled to the inner electrode for passing the electrical current through the inner electrode.  
50

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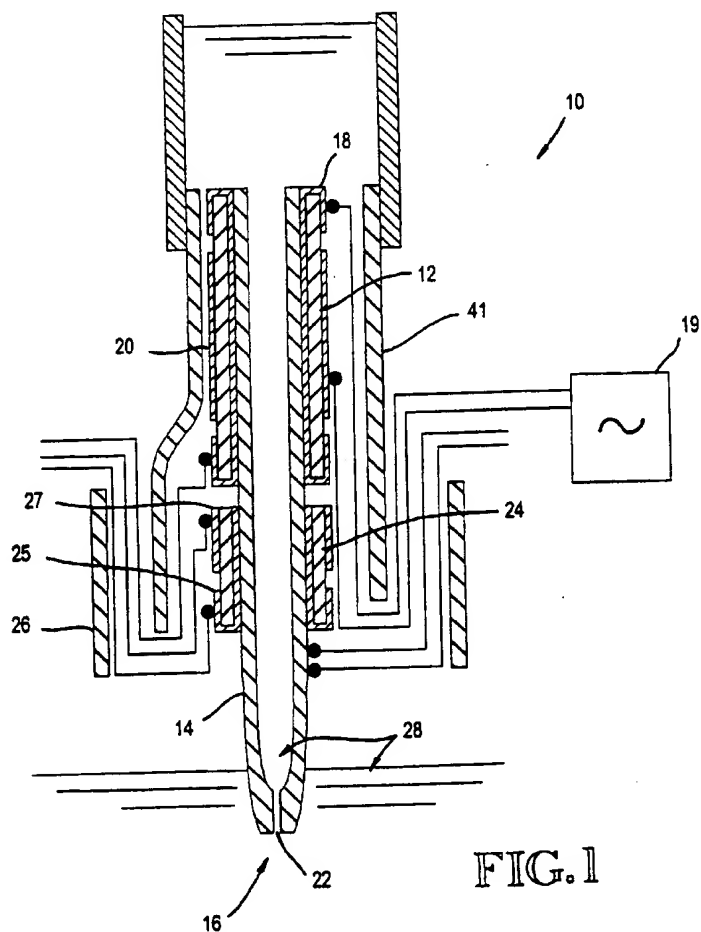


FIG. 1

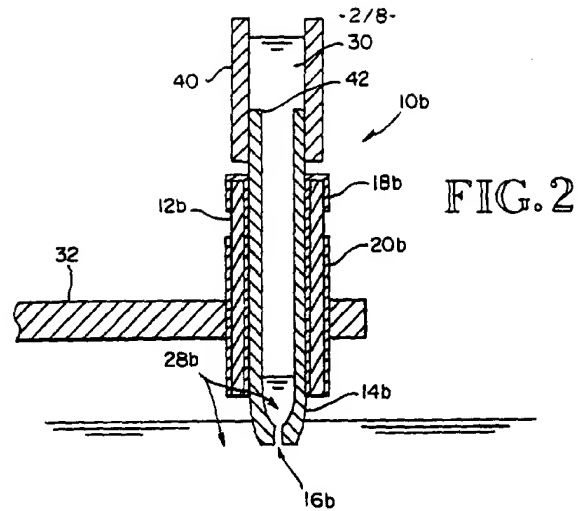


FIG. 2

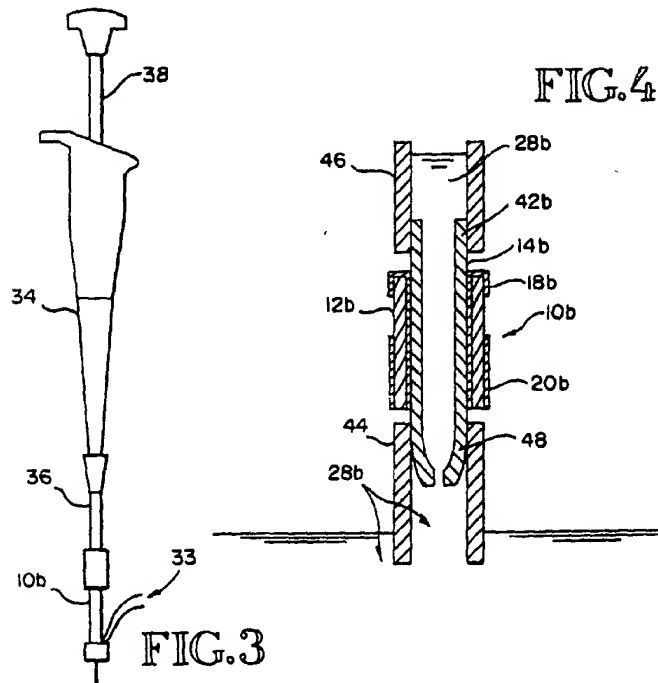
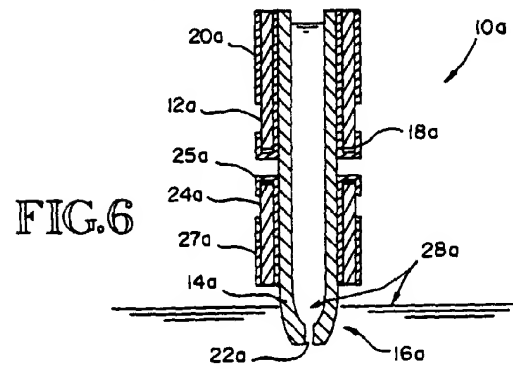
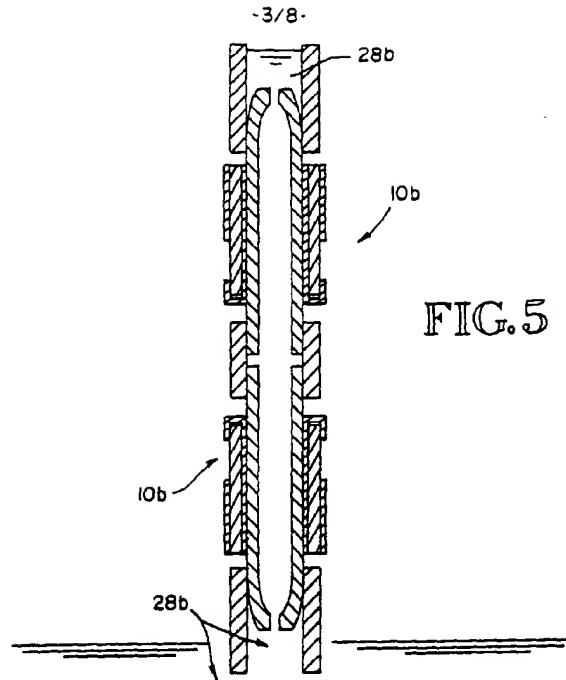


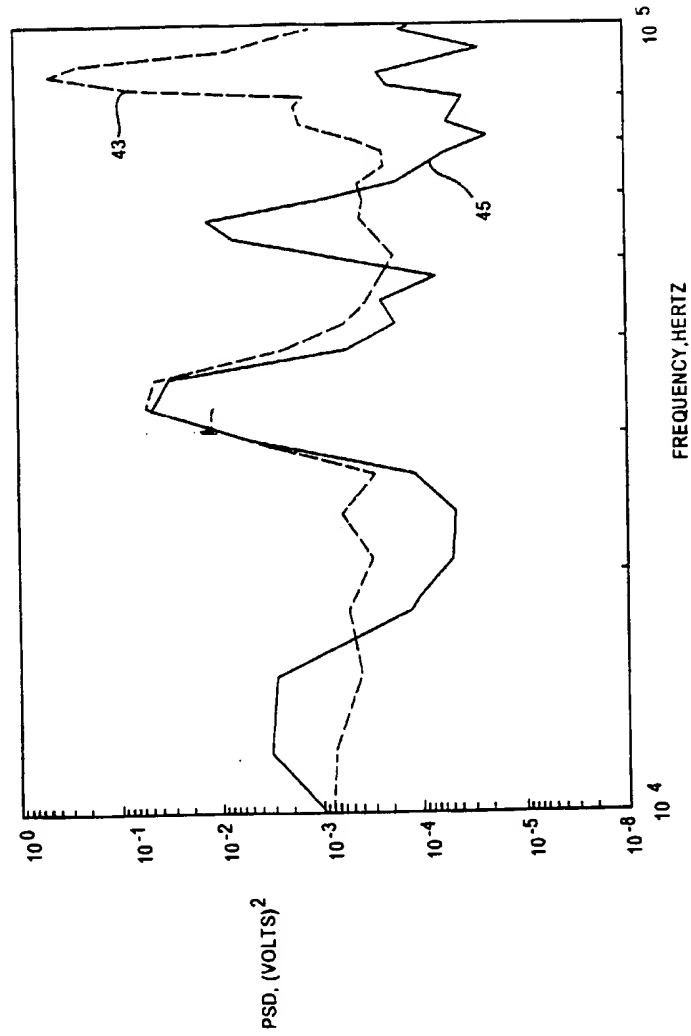
FIG. 4

FIG.3



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FIG. 7



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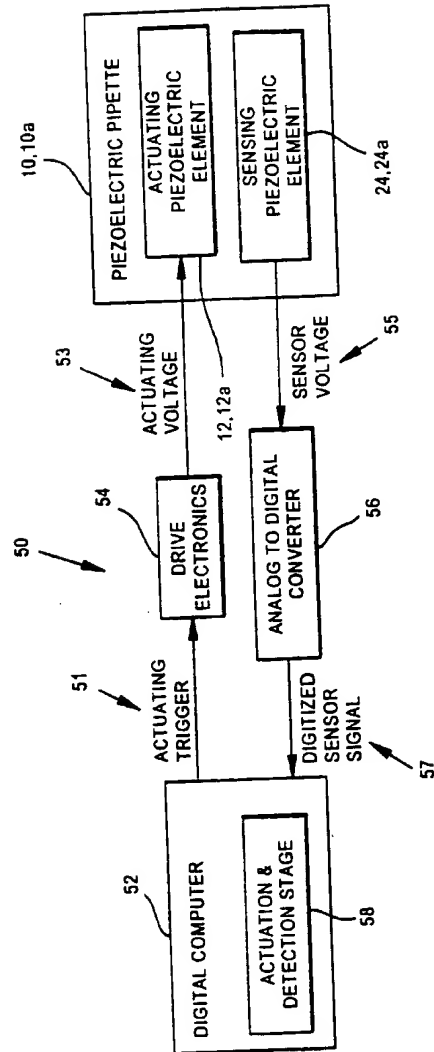
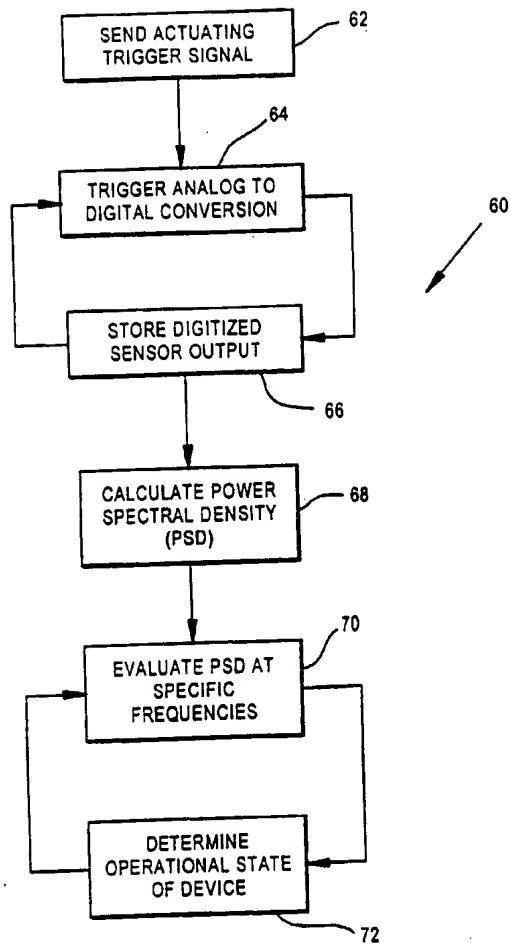


FIG. 8



FIG. 9



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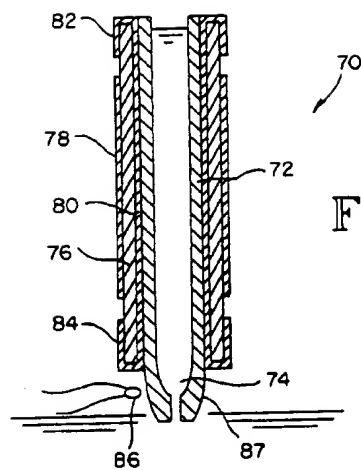
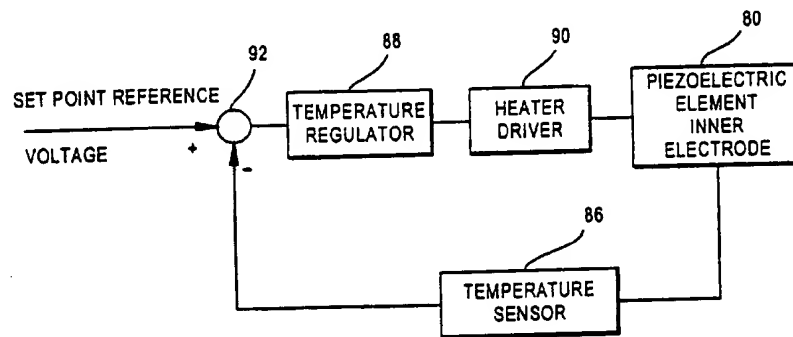


FIG. 11



# INTERNATIONAL SEARCH REPORT

Inter. nal Application No  
PCT/US 00/02730

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 B01L3/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 725 267 A (ROSSENDORF FORSCHZENT) 7 August 1996 (1996-08-07) abstract; figures 1-3 column 3, line 7 -column 5, line 3 column 5, line 49 -column 5, line 55	1-3,6
A		4,5,7,13
A	US 4 432 699 A (BECKMAN JOHN B ET AL) 21 February 1984 (1984-02-21) cited in the application abstract; figures 1-8 column 1, line 63 -column 2, line 40	4,5,7-12
A	WO 98 45205 A (PACKARD INSTRUMENT CO INC) 15 October 1998 (1998-10-15) figures 1,3 page 7, line 27 -page 8, line 13 page 11, line 22 -page 15, line 7	1,7,13
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

18 April 2000

Date of mailing of the International search report

27/04/2000

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/02730

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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